

What is Claimed Is:

39. A process for the preparation of L-threonine using bacteria of the Enterobacteriaceae family which produce L-threonine, comprising:

- 5           a) inoculating and culturing a bacterium of the Enterobacteriaceae family in at least a first nutrient medium;
- b) feeding at least one additional nutrient medium into the culture of step a) in one or more feed streams, wherein:
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          i) said additional nutrient medium comprises at least one source of carbon, at least one source of nitrogen and at least one source of phosphorus;

15           ii) said culture is maintained under conditions which allow the formation of L-threonine;

          iii) at the same that said additional nutrient medium is added, removing culture broth from the culture in one or several removal streams wherein the total flow rate of said one or more removal streams is substantially the same as the total flow rate of said one or more feed streams, and wherein

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- c) the concentration of the source of carbon during the continuous culturing in step b) is adjusted to not more than 30 g/l.

40. The process of claim 39, wherein the culturing in step (a) is carried out by a batch process.

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41. The process of claim 39, wherein the culturing of step (a) is carried out by a fed batch process, in which at least one additional nutrient medium is employed.
- 5 42. The process of claim 39, further comprising purifying L-threonine from said culture broth.
43. The process of claim 39, further comprising treating the culture broth removed in said one or several removal streams by:
- a) removing at least 90% of the biomass present; and
- 10 b) removing at least 90% of the water remaining in said removal stream after the removal of said biomass.
44. The process of claim 39, wherein said source of carbon is one or more compounds selected from the group
- 15 consisting of: sucrose; molasses from sugar beet or cane sugar; fructose; glucose; starch hydrolysate; cellulose hydrolysate; arabinose; maltose; xylose; acetic acid; ethanol; and methanol.
45. The process of claim 39, wherein said source of nitrogen is one or more organic nitrogen-containing substances or substance mixtures selected from the group consisting of: peptones; yeast extracts; meat extracts; malt
- 20 extracts; corn steep liquor; soya bean flour; and urea; and/or one or more of the inorganic compounds selected from the group consisting of: ammonia; ammonium-
- 25 containing salts; and salts of nitric acid.
46. The process of claim 39, wherein said source of nitrogen is selected from the group consisting of: ammonium sulfate; ammonium chloride; ammonium phosphate; ammonium
- 30 carbonate; ammonium nitrate; potassium nitrate; and potassium sodium nitrate.

47. The process of claim 39, wherein said source of phosphorus is either:

- a) phosphoric acid or polymers thereof; or
- b) phytic acid or alkali metal or alkaline earth metal salts thereof.

48. The process of claim 39, wherein said source of phosphorus is selected from the group consisting of: potassium dihydrogen phosphate; dipotassium hydrogen phosphate; and the corresponding sodium-containing salts thereof.

49. The process of claim 39, wherein said bacteria of the Enterobacteriaceae family contain at least one thrA gene or allele which codes for a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I.

50. The process of claim 39, wherein said bacteria of the Enterobacteriaceae family contains a stop codon selected from the group consisting of: opal; ochre; and amber; in the rpoS gene and a t-RNA suppressor selected from the group consisting of: opal suppressor; ochre suppressor; and amber suppressor.

51. The process of claim 39, wherein said nutrient feed medium has a phosphorus to carbon ratio (P/C ratio) selected from: not more than 4; not more than 3; not more than 2; not more than 1.5; not more than 1; not more than 0.7; not more than 0.5; not more than 0.48; not more than 0.46; not more than 0.44; not more than 0.42; not more than 0.40; not more than 0.38; not more than 0.36; not more than 0.34; not more than 0.32; and not more than 0.30.

52. The process of claim 39, wherein the culture broth removed is provided with oxygen or an oxygen-containing gas until the concentration of the source of carbon

falls below a value selected from: 2 g/l; 1 g/l; and 0.5 g/l.

53. The process of claim 50, wherein the L-threonine formed is purified.

5 54. The process of claim 50, further comprising treating the culture broth removed in said one or several removal streams by:

a) removing at least 90% of the biomass present; and

10 b) removing at least 90% of the water remaining in said removal stream after the removal of said biomass.

15 55. The process of claim 39, wherein the concentration of the source of carbon during the culture is adjusted to a value selected from: not more than 20; not more than 10; not more than 5 g/l and not more than 2 g/l.

56. The process of claim 39, wherein the yield of L-threonine formed, based on the source of carbon employed, is selected from a value of: at least 31%; at least 37%; and at least 42%.

20 57. The process of claim 39, wherein L-threonine is formed with a space/time yield having a value selected from: at least 1.5 to 2.5 g/l per h; 2.5 to more than 3.5 g/l per h; at least 3.5 to 5.0 g/l per h; at least 5.0 to more than 8.0 g/l per h.

25 58. Sucrose-utilizing transconjugants of *Escherichia coli* K-12 deposited as DSM 16293 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures] (Braunschweig, Germany).

59. The process of claim 39, wherein said bacteria of the Enterobacteriaceae family is a strain which has one or more of the following features:

- 5           a) a threonine-insensitive aspartate kinase I -  
homoserine dehydrogenase I, which is optionally  
present in overexpressed form;
- 10           b) a stop codon selected from the group consisting of:  
opal, ochre and amber in the rpoS gene; and a t-RNA  
suppressor selected from the group consisting of:  
opal suppressor, ochre suppressor and amber  
suppressor;

60. The process of claim 59, wherein said strain further comprises one or more of the following features:

- 15           a) an incapablitiy, under aerobic culture conditions,  
of breaking down threonine,
- b) at least a partial need for isoleucine, and
- c) a capacity to grow in the presence of at least 5  
g/l threonine.

61. The process of claim 59, wherein said strain further comprises one or more of the following features:

- 20           a) attenuation of phosphoenol pyruvate  
carboxykinase, which is coded for by the pckA  
gene;
- 25           b) attenuation of phosphoglucose isomerase, which is  
coded for by the pgk gene;
- c) attenuation of the YtfP gene product, which is  
coded for by the open reading frame ytfP;
- d) attenuation of the Yjfa gene product, which is  
coded for by the open reading frame yjfa;

- e) attenuation of pyruvate oxidase, which is coded for by the *poxB* gene;
- f) attenuation of the *YjgF* gene product, which is coded for by the open reading frame *yjgF*;
- 5 g) enhancement of transhydrogenase, which is coded for by the genes *pntA* and *pntB*;
- h) enhancement of phosphoenol pyruvate synthase, which is coded for by the *pps* gene;
- 10 i) enhancement of phosphoenol pyruvate carboxylase, which is coded for by the *ppc* gene;
- j) enhancement of the regulator *RseB*, which is coded for by the *rseB* gene;
- k) enhancement of the galactose proton symporter, which is coded for by the *galP* gene;
- 15 l) an ability to use sucrose as a source of carbon;
- m) enhancement of the *YedA* gene product, which is coded for by the open reading frame *yedA*;
- 20 n) growth in the presence of at least 0.1 to 0.5 mM or at least 0.5 to 1 mM borrelidin (borrelidin resistance);
- o) growth in the presence of at least 2 to 2.5 g/l or at least 2.5 to 3 g/l diaminosuccinic acid (diaminosuccinic acid resistance);
- 25 p) growth in the presence of at least 30 to 40 mM or at least 40 to 50 mM  $\alpha$ -methylserine ( $\alpha$ -methylserine resistance);
- q) growth in the presence of not more than 30 mM or not more than 40 mM or not more than 50 mM

fluoropyruvic acid (fluoropyruvic acid sensitivity);

5 r) growth in the presence of at least 210 mM or at least 240 mM or at least 270 mM or at least 300 mM L-glutamic acid (glutamic acid resistance);

s) at least a partial need for methionine;

t) at least a partial need for m-diaminopimelic acid;

10 u) growth in the presence of at least 100 mg/l rifampicin (rifampicin resistance);

v) growth in the presence of at least 15 g/l L-lysine (lysine resistance);

15 w) growth in the presence of at least 15 g/l methionine (methionine resistance);

x) growth in the presence of at least 15 g/l L-aspartic acid (aspartic acid resistance); or

y) enhancement of pyruvate carboxylase, which is coded for by the pyc gene.